

New Delhi metallo- β -lactamase-producing *Acinetobacter baumannii*: a novel paradigm for spreading antibiotic resistance genes

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ABSTRACT: The impact of carbapenemase production among clinically significant Gram-negative rods is becoming a major medical issue. To date, *Acinetobacter baumannii* has been considered as a final recipient of carbapenemase genes (imipenemase, Verona metallo- β -lactamase, Guiana extended-spectrum β -lactamase and *Klebsiella pneumonia* carbapenemase types) from Enterobacteriaceae and *Pseudomonas aeruginosa*. However, recent findings regarding the spread of the *bla*_{NDM} carbapenemase genes revealed that *A. baumannii* likely acts as a source of emerging antibiotic resistance genes. The analysis of genetic structure surrounding the *bla*_{NDM-1} gene revealed that the genetic structure (Tn125) responsible for its dissemination most probably originates from *Acinetobacter*. Moreover, analysis of the *bla*_{NDM-1} gene itself demonstrated that it might be constructed in *Acinetobacter* through a recombination event with another resistance gene found in *A. baumannii* (*aphA6*). This novel paradigm highlights a novel and unexpected role played by *A. baumannii*.

Members of the genus *Acinetobacter* seem to have the ability to quite rapidly acquire resistance to new antibiotics [1]. The rise of resistance to broad-spectrum antibiotics such as aminoglycosides, expanded-spectrum cephalosporins, carbapenems and tigecycline in *Acinetobacter baumannii* has left few therapeutic options [2]. The main problem corresponds to the rise of carbapenem resistance in *A. baumannii*, which is almost always identified in multidrug-resistant clinical isolates [3]. Until now, *A. baumannii* has been considered a final reservoir of antibiotic resistance genes by acquisition of foreign DNA from different sources, particularly from other Gram-negative species. Many resistance genes have been identified in *A. baumannii* including those encoding extended-spectrum β -lactamases (PER-, Guiana extended-spectrum β -lactamase [GES] and VEB type) and aminoglycoside resistance genes [1,4]. The acquisition of those genes is often related to an integration of foreign DNA originating from other clinical species (Enterobacteriaceae and *Pseudomonas aeruginosa*) [1,2,4–7].

Carbapenem resistance in *A. baumannii*

Genes encoding metallo- β -lactamases of verona metallo- β -lactamase- and imipenemase types, as well as those encoding class A carbapenemases (*Klebsiella pneumonia* carbapenemase or GES), are other examples of acquisition of foreign resistance genes as a source of carbapenem resistance in *A. baumannii* [2]. Analysis of the genetic structures surrounding those carbapenemase genes reveals that they are not specific to *Acinetobacter*. The identified genetic structures argue for a transfer

KEYWORDS

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from Enterobacteriaceae and *P. aeruginosa* to *A. baumannii*.

The most important source of carbapenem resistance in *A. baumannii* is the production of carbapenem-hydrolyzing Ambler class D β -lactamases (CHDL) [7]. Five different subgroups of acquired CHDLs have been identified, namely oxacillinase, class D β -lactamase (OXA)-23, -40, -58, -143 and -235 [2,7,8]. These five CHDL groups are specific to the *Acinetobacter* species and are not usually identified in other clinically relevant species, such as in Enterobacteriaceae and in *P. aeruginosa* [2,7]. In contrast to what is observed for other types of carbapenemase genes (those encoding the carbapenemases *K. pneumonia* carbapenemase, GES, verona metallo- β -lactamase and imipenemase), CHDL-encoding genes are associated with insertion sequences (IS) identified in *Acinetobacter* species (e.g., IS*Aba1*). The natural progenitor of one of these carbapenemase subgroups (OXA-23) has been identified as being *Acinetobacter radioresistens* [9]. To date, this corresponds to one of the few examples of a resistance gene originating from an *Acinetobacter* species and targeting another *Acinetobacter* species.

The case of the *bla*_{NDM} genes

The *bla*_{NDM-1} gene is one of the latest carbapenemase genes to have been reported. They have now been extensively reported in Enterobacteriaceae (*Escherichia coli* and *K. pneumoniae*), first from India, Pakistan and Bangladesh, and then from the rest of the world [10–12]. The occurrence of the *bla*_{NDM-1} gene in the environment has also been evidenced in India, particularly among environmental Gram-negative species [13]. Sequencing of a *bla*_{NDM-1}-bearing plasmid, as part of the analysis of the resistosome of a multidrug-resistant *E. coli*, led to the identification of a remnant of IS*Aba125*, located immediately upstream of the *bla*_{NDM-1} gene [14]. The IS*Aba125* element provided the -35 sequence of the hybrid promoter responsible for the expression of the *bla*_{NDM-1} gene [14]. Interestingly, this IS element had been originally identified from an *A. baumannii* isolate (hence its name) without any physical association with the *bla*_{NDM} gene [15]. Following this, IS*Aba125* was identified in many other *A. baumannii* isolates [6,16]. By contrast, this IS has been identified in Enterobacteriaceae and *P. aeruginosa* only as a remnant of the Tn*125* originating from *Acinetobacter* species and has never been identified alone in these species [17].

This observation suggests that *A. baumannii* is a likely reservoir of IS*Aba125*.

While the first studies dealt with the *bla*_{NDM}-like genes from enterobacterial isolates, many *A. baumannii* isolates carrying the *bla*_{NDM}-like gene have been now identified (Table 1) [2,18]. The possible progenitor (still not identified) of the *bla*_{NDM} gene is considered to be phylogenetically distant from *A. baumannii* owing to the guanine-cytosine content of this gene (62% for the *bla*_{NDM} gene vs from 38 [Acinetobacter calcoaceticus] up to 42% [Acinetobacter lwoffii] for genomes of *Acinetobacter* species). Likewise, the guanine-cytosine content of IS*Aba125* is 37% and the fact that IS*Aba125* has been identified alone (not associated with the *bla*_{NDM-1} gene) seems to indicate the occurrence of independent acquisition of *bla*_{NDM-1} and IS*Aba125*. Transfer of the *bla*_{NDM-1} gene to *A. baumannii* is probably the result of a transfer from an unknown bacterial species to *A. baumannii*, with both donor and recipient probably being present concomitantly in a same environment. In *A. baumannii*, the *bla*_{NDM-1} gene is part of a 10,099-bp composite transposon made of two copies of IS*Aba125* (Figure 1) [19]. Downstream of *bla*_{NDM-1}/*bla*_{NDM-2}, eight open reading frames have been identified (Figure 1). The first corresponds to the *ble*_{MBL} gene, encoding a 121-amino acid-long protein conferring resistance to bleomycin (anticancer/antibiotic drug), previously found associated with *bla*_{NDM-1} in enterobacterial isolates [20]. Then, several genes encoding putative proteins sharing similarities with genes identified from the *Brevundimonas* and *Xanthomonas* genera were identified [19]. Downstream, two genes encoding the GroES and GroEL chaperonin proteins, respectively, were identified. Finally, a gene encoding the putative transposase of an IS common region (ISCR)-like element was identified sharing 93% protein sequence identity with ISCR19 [21]. ISCR elements are IS able to mobilize DNA fragment located at their left-hand extremity by rolling-circle transposition [22]. At the right-hand extremity of ISCR21 and before the second copy of IS*Aba125* of Tn*125*, a truncated gene encoding a putative phospholipid acetyltransferase was identified, with the corresponding protein sequence sharing 91% amino acid identity with that of *Acinetobacter junii* (Figure 1) [19]. This finding indicates a likely intermediate state that may have occurred in an *Acinetobacter* species other than *A. baumannii*. Our hypothesis is

Table 1. Epidemiologic features of NDM-producing *Acinetobacter* species.

Isolates or species	Isolates (n)	Acquired carbapenemase	Worldwide clone	MLST analysis [†]	Country of isolation	Probable origin	Ref.
<i>Acinetobacter baumannii</i> isolates							
<i>A. baumannii</i> JH	1	NDM-1, OXA-23	WWI	ST1 ^P	Switzerland	Balkans region	[18]
<i>A. baumannii</i> ANC4097	1	NDM-1, OXA-23	WWI	ST1 ^P	Czech Republic	Egypt	[40]
<i>A. baumannii</i> 161/07	1	NDM-1	None	ST25 ^P	Germany	Serbia	[41]
<i>A. baumannii</i> Slo	1	NDM-1	None	ST25 ^P	Slovenia	Balkans region	[18]
<i>A. baumannii</i>	8	NDM-1	None	ST85 ^P	France	North Africa	[32]
<i>A. baumannii</i> Ab11314	1	NDM-1	WWII	ST92 ^B	Belgium	Algeria	[42]
<i>A. baumannii</i> A28, A32 and A36	3	NDM-1, OXA-23	N/A	N/A	India	India	[26]
<i>A. baumannii</i>	1	NDM-1, OXA-23	N/A	N/A	Japan	India	[43]
<i>A. baumannii</i> ABCA207, ABC3229, ABC4289, ABWA7	4	NDM-1	N/A	N/A	China	China	[27]
<i>A. baumannii</i> ML	1	NDM-2	None	ST103 ^P	Germany	Egypt	[33]
<i>A. baumannii</i> I1, I2, I15, I16, I17	5	NDM-2	None	ST103 ^P	Israel	?	[34]
<i>A. baumannii</i> 124, 132	2	NDM-2	None	ST103 ^P	United Arab Emirates	United Arab Emirates	[35]
<i>Acinetobacter non-baumannii</i> isolates							
<i>A. lwoffii</i> WJ10621	1	NDM-1	N/A	N/A	China	?	[44]
<i>A. pittii</i> D499	1	NDM-1	N/A	N/A	China	?	[45]
<i>A. junii</i> 1454	1	NDM-1	N/A	N/A	China	?	[46]
<i>Acinetobacter</i> spp.	9	NDM-1	N/A	N/A	China	China	[28]
<i>A. lwoffii</i> SGC-HZ9	1	NDM-1	N/A	N/A	China	China	[29]
<i>A. pittii</i>	27	NDM-1	N/A	ST63 ^P	China	China	[47]
<i>A. johnsonii</i>	2	NDM-1	N/A	N/A	China	China	[30]

[†]Two different MLST schemes are currently available for the typing of *Acinetobacter* species. Here, ST^P designed Pasteur's Institute MLST scheme and ST^B designed Bartual and colleagues MLST scheme.
MLST: Multilocus sequence typing; N/A: Not applicable; NDM: New Delhi metallo-β-lactamase; OXA: Oxacillinase, class D β-lactamase; ST: Sequence type; WWI: Worldwide clonal lineage I; WWII: Worldwide clonal lineage II.

that an *Acinetobacter* species acquired the *bla*_{NDM} gene from an environmental species, probably via a natural transformation process (a physiological process encoded by a wide range of bacteria, permitting the uptake of exogenous DNA via the binding of dsDNA on a specific membrane receptor followed by the entry of ssDNA; finally, homologous recombination is necessary to incorporate the exogenous DNA) (Figure 2). In that species, the transposon Tn125 was built. Subsequently, *A. baumannii* acquired the *bla*_{NDM} gene through the acquisition of this transposon. After its dissemination among *Acinetobacter* species, an interspecies transfer via a broad-host range plasmid permitted the acquisition of Tn125 by Enterobacteriaceae or *P. aeruginosa*. This step was followed by a large dissemination

of the *bla*_{NDM} in Enterobacteriaceae (Figure 2).

Interestingly, a recent study suggested that the *bla*_{NDM-1} has been constructed in *Acinetobacter* [23]. Precise genetic analysis of the *bla*_{NDM-1} gene itself revealed an interesting feature. It shared the 5' end sequence of the aminoglycoside resistance gene *aphA6* (displaying 100% identity in the spacer sequence [260 bp] between IS*Aba125* and either the *bla*_{NDM-1} or *aphA6* genes and the first 20 nucleotides of the both genes) [23]. Figure 1B demonstrates the two hypotheses of the construction of the *bla*_{NDM-1} into *Acinetobacter* species:

- The first hypothesis is a homologous recombination process between, on the one hand, a composite transposon made of two copies of IS*Aba125* and carrying the *aphA6* gene, and,

Figure 1. Diversity of genetic structures surrounding the bla_{NDM-1} gene (facing page). (A) Genetic analysis comparison of the structures identified in *Acinetobacter baumannii* and other Gram-negative rods: Tn125 (Genbank accession number JN872329) from *A. baumannii* NRZ [19], pKP-NDM (Genbank accession number JN157804) from *Klebsiella pneumoniae* Kp7 [48], pNDM-GUE (Genbank accession number JQ364967) from *Escherichia coli* GUE [17] and p271A (Genbank accession number JF785549) from *E. coli* 271 [14]. Gene name abbreviations are deduced from their corresponding proteins. Insertion sequence elements are ISAbt125, ISCR21, ISKpn7, IS26, ISEC33 and ISSen4, whereas transposon is Tn5403. *aadA2* and *drfA17* encode aminoglycoside and trimethoprim resistance, respectively. Base pair duplications are indicated by 3 and 8 bp. The *oriIS* of ISCR21 is indicated by a circle. The conserved bla_{NDM-1} locus is indicated by vertical black lines. (B) Possible hypotheses of the bla_{NDM-1} construction: hypothesis 1: the bla_{NDM-1} gene could have been constructed by a recombination event removing the sequence between the arrows, including the *aphA6* gene, and giving rise to the bla_{NDM-1} gene; hypothesis 2: the bla_{NDM-1} could have been constructed by rolling-circle transposition mediated by ISCR21 into the *aphA6* gene. The yellow box into the bla_{NDM-1} gene indicates the shared sequence between the bla_{NDM-1} and the *aphA6* genes. IRR: Inverted repeat right; IRL: Inverted repeat left; IS: Insertion sequence; ISCR: Insertion sequence common region.

on the other hand, the bla_{NDM-1} gene, giving rise to the neoformed bla_{NDM-1} gene identified in the Tn125;

- The construction was due to two steps of the rolling-circle transposition event, which inserted the fragment containing the bla_{NDM-1} gene from the progenitor into the *aphA6* gene (Figure 1B).

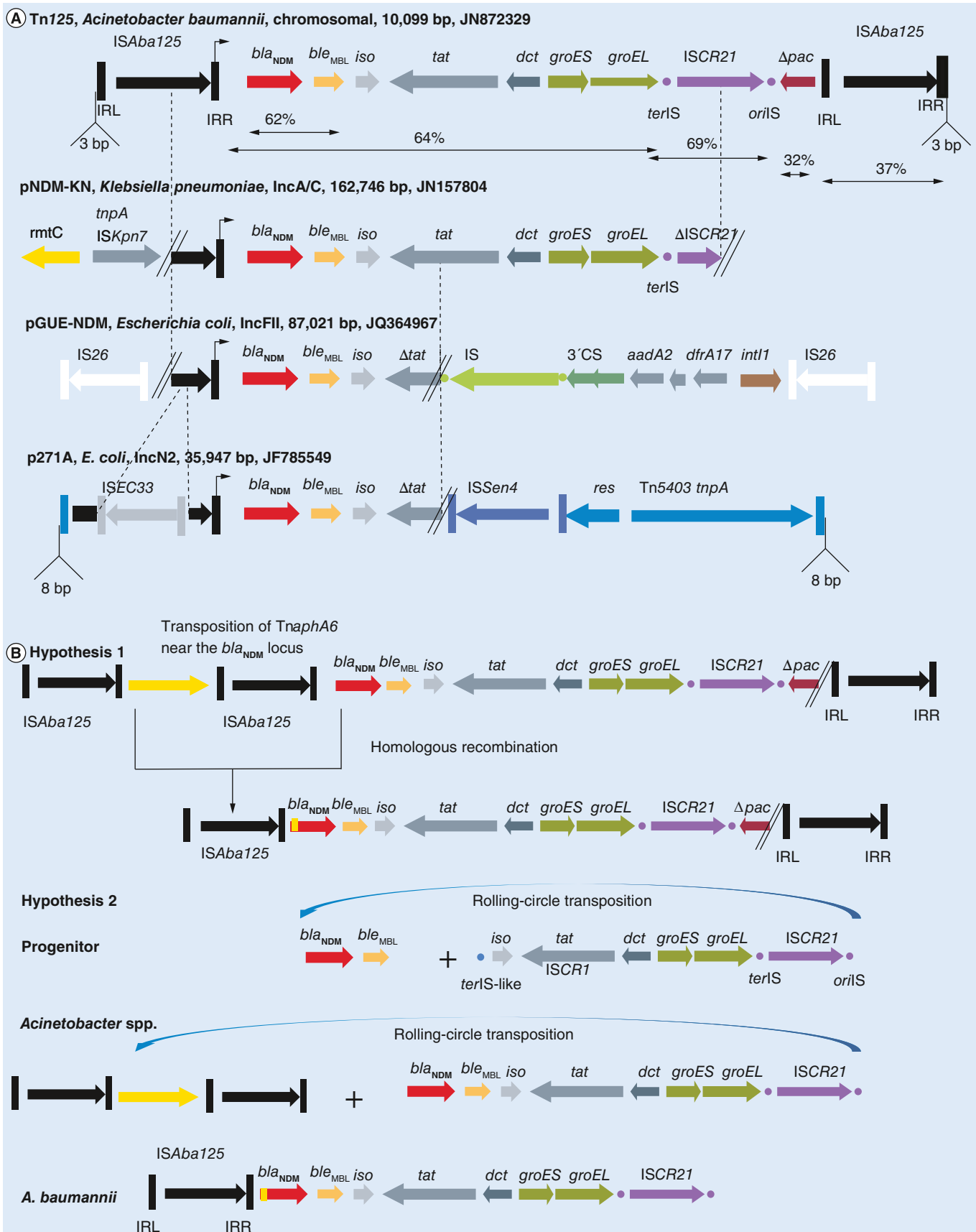
In light of these recent findings, a new role for *A. baumannii* may be considered. Although usually considered as a final recipient of resistance genes, *A. baumannii* may transfer them to Enterobacteriaceae, and *P. aeruginosa* after a probable acquisition from an environmental resistome (Figure 2). To date, the role of *A. baumannii* as a source of resistance genes for other clinically significant bacterial species has not been underlined. There is only one single report of the bla_{OXA-23} gene outside *A. baumannii*, being identified in *Proteus mirabilis*, as well as another single report of acquisition of the natural cephalosporinase bla_{ADC} gene in *Oligella urethralis* (a Gram-negative rod belonging to Moraxellaceae [24,25]). However, in these two cases, *A. baumannii* has played the role of progenitor, whereas it played the role of an intermediate reservoir for the bla_{NDM} genes.

Epidemiology of NDM-producing *Acinetobacter* species

NDM-1-producing *A. baumannii* were first identified in India [26] and then in China [27]. NDM-1-producing non-*baumannii* *Acinetobacter*, including *A. lwoffii*, *A. junii*, *Acinetobacter pittii*, *Acinetobacter haemolyticus* and *Acinetobacter* genomospecies 10 and 15, have been reported from nosocomial settings and environmental sources in China [28–30]. In these studies, the bla_{NDM-1} gene is carried by a plasmid with a size ranging from approximately 30 to 55 kb,

which may be transferred to *E. coli* by conjugation. Although these experiments are *in vitro* analyses, we might hypothesize that the dissemination of the bla_{NDM} genes from *Acinetobacter* species to Enterobacteriaceae could be due to the interspecies transfer of this plasmid. In contrast to what is observed in China, a report highlighting the scattered diffusion of NDM-1-producing *A. baumannii* in Europe demonstrated that this dissemination was neither due to a single clone nor to plasmid diffusion, but rather to a spread of different clones carrying a same transposon Tn125 [18]. Multilocus sequence typing (MLST) analysis using Pasteur's Institute scheme [31] revealed that the clones circulating in Europe belonged to sequence type (ST)1 (also named worldwide clone I) and ST25 with a probable link with the Balkans region. Recently, several *A. baumannii* clinical isolates possessing the bla_{NDM-1} gene and belonging to ST85 according to Pasteur's Institute MLST scheme have been identified. These isolates were recovered from patients originating from North Africa, with no obvious link to the Indian subcontinent. This finding strongly suggests that a single NDM-producing *A. baumannii* clone is likely widespread in North Africa and that it may act as a secondary reservoir of spread of the NDM-1 resistance trait [32]. These genome comparison analyses indicated that several reservoirs of NDM-1-producing *A. baumannii* have been established at least in Asia (China and India), the Balkans region and North Africa.

Hitherto, only a single NDM-1 variant has been described in *A. baumannii*, NDM-2 [33]. This variant has been identified in a German patient (with a travel history in Egypt), from Israel and from the United Arab Emirates [33–35]. MLST analysis revealed that these isolates belong to the same clone, ST103 (or ST253 according to MLST scheme developed by Bartual and



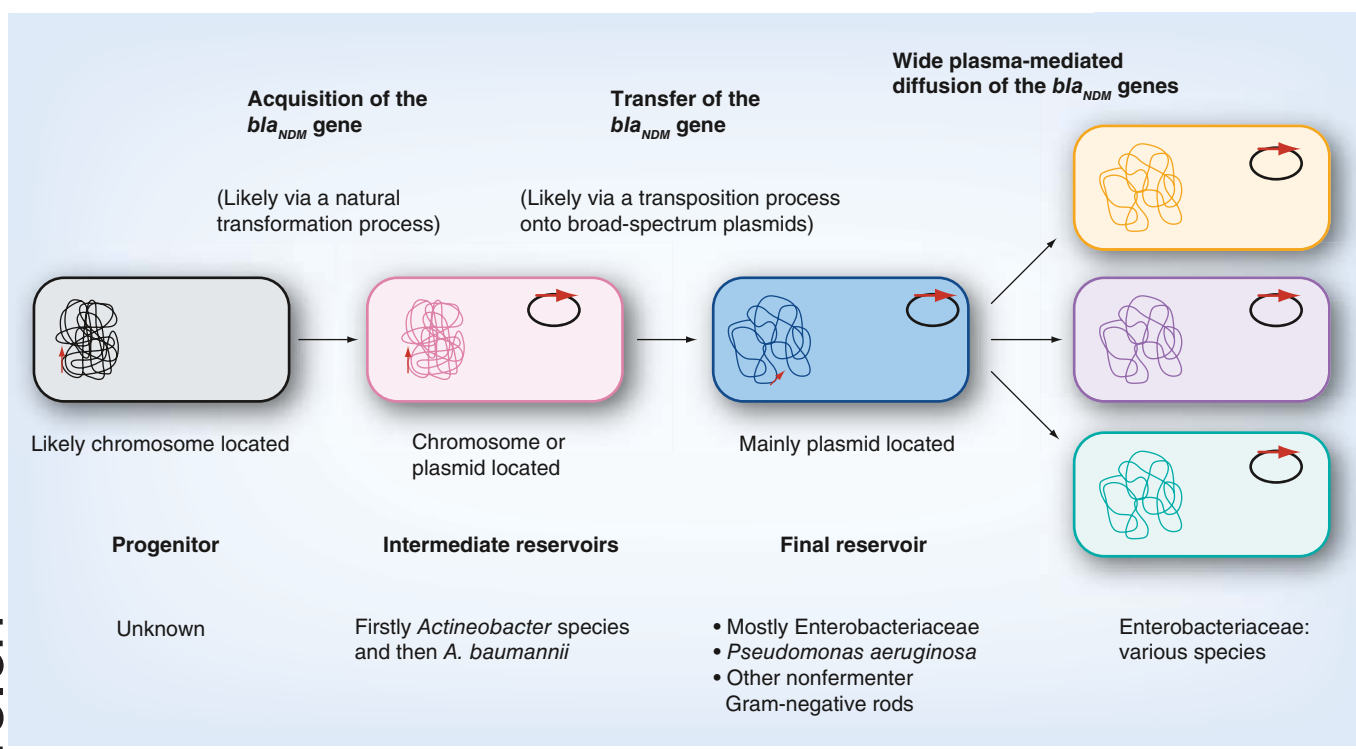


Figure 2. Hypothesis of the diffusion of the bla_{NDM} genes from the progenitor to clinical isolates. The different species are indicated by different colors.

colleagues [36]), corresponding to a new clone with no link to sequence types found elsewhere. In contrast to NDM-1-producing *A. baumannii*, the reservoir of the bla_{NDM-2} gene seems to be present at least in the Middle East.

Medical implications

Identification of *A. baumannii* as a source of multidrug resistance is now well established. We suggest that identification of resistance genes in *A. baumannii* worldwide is important, not only for preventing the spread of multidrug resistance traits in *A. baumannii*, but also for preventing their potential transfer to Enterobacteriaceae, which are by far the most important sources of infection in humans. *A. baumannii*, which had been mostly considered a weak pathogen responsible for infections in immunocompromized patients, may become a Trojan horse for spreading antibiotic resistance genes in unrelated and clinically significant Gram-negative species.

More specifically, identification of NDM producers in *A. baumannii* appears to be crucial considering that these isolates have already disseminated at least in some areas (North Africa, Europe, China and India).

Another important concern is that *A. baumannii* is well-known to persist much longer than Enterobacteriaceae in the environment [37]. Consequently, outbreaks involving multidrug-resistant *A. baumannii* are difficult to eradicate [1,2,38], as exemplified by a study demonstrating the re-emergence of the same *A. baumannii* clone 6 months later in a burns unit owing to environmental contamination [39]. The closure of this burns unit was necessary to eradicate the contamination [39]. Early identification is therefore very important.

Future perspective

The current diffusion of carbapenemase genes in Gram-negative rods is now a serious public health issue and, in the near future, could lead back to the preantibiotic era. The screening of multidrug-resistant *A. baumannii* and the monitoring, in particular, of the carbapenem resistance in that species has to be considered a crucial issue. The NDM saga demonstrates that the diffusion of the bla_{NDM-1} gene in Enterobacteriaceae, probably originating from *Acinetobacter* species, shows that diffusion of resistance genes is more complex than expected.

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EXECUTIVE SUMMARY

Background

- Members of the genus *Acinetobacter* have the ability to rapidly develop resistance to new antibiotics.
- The main problem concerning antibiotic resistance in *Acinetobacter baumannii* corresponds to the rise of carbapenem resistance, which is always associated with multidrug resistance.
- *A. baumannii* has been considered as a final recipient of antibiotic resistance genes originating from other clinically relevant Gram-negative species.

Carbapenem resistance in *A. baumannii*

- Carbapenem-hydrolyzing Ambler class D β -lactamases are the main source of carbapenem resistance in *A. baumannii*.
- The acquisition of most Ambler class B and A carbapenemases has resulted from the integration of foreign DNA in *A. baumannii* from Enterobacteriaceae or *Pseudomonas aeruginosa*.

The case of the *bla*_{NDM} genes

- The *bla*_{NDM} genes are associated with the insertion sequence *Aba125* (often truncated in Enterobacteriaceae), which plays a role in *bla*_{NDM} expression.
- In *A. baumannii*, the *bla*_{NDM-1} gene is part of a 10,099-bp composite transposon made of two copies of *ISAba125*.
- A new role may be played by *A. baumannii* involving the acquisition of the *bla*_{NDM-1} resistance gene and then transferring it to Enterobacteriaceae and *P. aeruginosa*.

Epidemiology of NDM-producing *Acinetobacter* species

- NDM-1-producing *A. baumannii* has been widely identified in India and China from nosocomial settings and environmental sources.
- Whereas the diffusion of NDM-1-producing *A. baumannii* in Asia seems to be due to a plasmid diffusion, in Europe, it was neither due to a single clone nor any plasmid diffusion, but rather to different clones carrying the transposon Tn125.
- Multilocus sequence typing analyses indicated several reservoirs of NDM-1-producing *A. baumannii* including Asia (mainly China and India), the Balkans region and North Africa.

Medical implication

- The identification of resistance genes in *A. baumannii* worldwide is important not only for preventing the spread of multidrug resistance in *A. baumannii*, but also for preventing their extension to Enterobacteriaceae.
- Outbreaks involving multidrug-resistant *A. baumannii* are known to be very difficult to eradicate owing to the ability of this bacterial species to persist in a nosocomial environment.

References

Papers of special note have been highlighted as:

- of interest
- of considerable interest

1 Peleg AY, Seifert H, Paterson DL. *Acinetobacter baumannii*: emergence of a successful pathogen. *Clin. Microbiol. Rev.* 21(3), 538–582 (2008).

- Important review on the biology of *Acinetobacter*.

2 Poirel L, Bonnin RA, Nordmann P. Genetic basis of antibiotic resistance in pathogenic *Acinetobacter* species. *IUBMB Life* 63(12), 1061–1067 (2011).

3 Fishbain J, Peleg AY. Treatment of *Acinetobacter* infections. *Clin. Infect. Dis.* 51(1), 79–84 (2010).

- Interesting review on the treatment of multidrug-resistant *Acinetobacter*.

4 Poirel L, Bonnin RA, Nordmann P. Genetic support and diversity of acquired extended-spectrum β -lactamases in Gram-negative rods. *Infect. Genet. Evol.* 12(5), 883–893 (2012).

- Up-to-date review on the genetic of extended-spectrum β -lactamase acquisition.

5 Fournier PE, Vallenet D, Barbe V *et al.* Comparative genomics of multidrug resistance in *Acinetobacter Baumannii*. *PLoS Genet.* 2(1), e7 (2006).

- 6 Iacono M, Villa L, Fortini D *et al.* Whole-genome pyrosequencing of an epidemic multidrug-resistant *Acinetobacter baumannii* strain belonging to the European clone II group. *Antimicrob. Agents Chemother.* 52(7), 2616–2625 (2008).
- 7 Poirel L, Naas T, Nordmann P. Diversity, epidemiology, and genetics of class D β -lactamases. *Antimicrob. Agents Chemother.* 54(1), 24–38 (2010).
- 8 Higgins PG, Perez-Llarena FJ, Zander E *et al.* OXA-235, a novel class D β -lactamase involved in resistance to carbapenems in *Acinetobacter baumannii*. *Antimicrob. Agents Chemother.* 57(5), 2121–2126 (2013).
- 9 Poirel L, Figueiredo S, Cattoir V, Carattoli A, Nordmann P. *Acinetobacter radioresistens* as a silent source of carbapenem resistance for *Acinetobacter* spp. *Antimicrob. Agents Chemother.* 52(4), 1252–1256 (2008).
- 10 Kumarasamy KK, Toleman MA, Walsh TR *et al.* Emergence of a new antibiotic resistance mechanism in India, Pakistan, and the UK: a molecular, biological, and epidemiological study. *Lancet Infect. Dis.* 10(9), 597–602 (2010).
- **First nationwide survey on the spread of the bla_{NDM-1} gene.**
- 11 Nordmann P, Poirel L, Walsh TR, Livermore DM. The emerging NDM carbapenemases. *Trends Microbiol.* 19(12), 588–595 (2011).
- 12 Yong D, Toleman MA, Giske CG *et al.* Characterization of a new metallo- β -lactamase gene, bla_{NDM-1} , and a novel erythromycin esterase gene carried on a unique genetic structure in *Klebsiella pneumoniae* sequence type 14 from India. *Antimicrob. Agents Chemother.* 53(12), 5046–5054 (2009).
- **The first identification of the bla_{NDM-1} gene.**
- 13 Walsh TR, Weeks J, Livermore DM, Toleman MA. Dissemination of NDM-1 positive bacteria in the New Delhi environment and its implications for human health: an environmental point prevalence study. *Lancet Infect. Dis.* 11(5), 355–362 (2011).
- **Study of the spread of the bla_{NDM-1} gene in the environment in New Dehli.**
- 14 Poirel L, Bonnin RA, Nordmann P. Analysis of the resistome of a multidrug-resistant NDM-1-producing *Escherichia coli* strain by high-throughput genome sequencing. *Antimicrob. Agents Chemother.* 55(9), 4224–4229 (2011).
- 15 Mussi MA, Limansky AS, Viale AM. Acquisition of resistance to carbapenems in multidrug-resistant clinical strains of *Acinetobacter baumannii*: natural insertional inactivation of a gene encoding a member of a novel family of β -barrel outer membrane proteins. *Antimicrob. Agents Chemother.* 49(4), 1432–1440 (2005).
- 16 Lopes BS, Amyes SG. Role of IS*Aba1* and IS*Aba125* in governing the expression of bla_{ADC} in clinically relevant *Acinetobacter baumannii* strains resistant to cephalosporins. *J. Med. Microbiol.* 61(Pt 8), 1103–1108 (2012).
- 17 Bonnin RA, Poirel L, Carattoli A, Nordmann P. Characterization of an IncFII plasmid encoding NDM-1 from *Escherichia coli* ST131. *PLoS ONE* 7(4), e34752 (2012).
- 18 Bonnin RA, Poirel L, Naas T *et al.* Dissemination of New Delhi metallo- β -lactamase-1-producing *Acinetobacter baumannii* in Europe. *Clin. Microbiol. Infect.* 18(9), e362–e365 (2012).
- 19 Poirel L, Bonnin RA, Boulanger A *et al.* Tn125-related acquisition of bla_{NDM-1} -like genes in *Acinetobacter baumannii*. *Antimicrob. Agents Chemother.* 56(2), 1087–1089 (2012).
- 20 Dortet L, Nordmann P, Poirel L. Association of the emerging carbapenemase NDM-1 with a bleomycin resistance protein in Enterobacteriaceae and *Acinetobacter baumannii*. *Antimicrob. Agents Chemother.* 56(4), 1693–1697 (2012).
- 21 Naas T, Namdari F, Bogaerts P *et al.* Genetic structure associated with bla_{OXA-18} , encoding a clavulanic acid-inhibited extended-spectrum oxacillinase. *Antimicrob. Agents Chemother.* 52(11), 3898–3904 (2008).
- 22 Toleman MA, Bennett PM, Walsh TR. ISCR elements: novel gene-capturing systems of the 21st century? *Microbiol. Mol. Biol. Rev.* 70(2), 296–316 (2006).
- 23 Toleman MA, Spencer J, Jones L, Walsh TR. bla_{NDM-1} is a chimera likely constructed in *Acinetobacter baumannii*. *Antimicrob. Agents Chemother.* 56(5), 2773–2776 (2012).
- 24 Bonnet R, Marchandin H, Chanal C *et al.* Chromosome-encoded class D β -lactamase OXA-23 in *Proteus mirabilis*. *Antimicrob. Agents Chemother.* 46(6), 2004–2006 (2002).
- 25 Mammeri H, Poirel L, Mangeney N, Nordmann P. Chromosomal integration of a cephalosporinase gene from *Acinetobacter baumannii* into *Oligella urethralis* as a source of acquired resistance to β -lactams. *Antimicrob. Agents Chemother.* 47(5), 1536–1542 (2003).
- 26 Karthikeyan K, Thirunarayan MA, Krishnan P. Coexistence of bla_{OXA-23} with bla_{NDM-1} and *armA* in clinical isolates of *Acinetobacter baumannii* from India. *J. Antimicrob. Chemother.* 65(10), 2253–2254 (2010).
- **First description of the bla_{NDM-1} gene in *A. baumannii*.**
- 27 Chen Y, Zhou Z, Jiang Y, Yu Y. Emergence of NDM-1-producing *Acinetobacter baumannii* in China. *J. Antimicrob. Chemother.* 66(6), 1255–1259 (2011).
- 28 Fu Y, Du X, Ji J *et al.* Epidemiological characteristics and genetic structure of bla_{NDM-1} in non-*baumannii* *Acinetobacter* spp. in China. *J. Antimicrob. Chemother.* 67(9), 2114–2122 (2012).
- 29 Wang Y, Wu C, Zhang Q *et al.* Identification of New Delhi metallo- β -lactamase 1 in *Acinetobacter lwoffii* of food animal origin. *PLoS ONE* 7(5), e37152 (2012).
- 30 Zong Z, Zhang X. bla_{NDM-1} -carrying *Acinetobacter johnsonii* detected in hospital sewage. *J. Antimicrob. Chemother.* 68(5), 1007–1010 (2013).
- 31 Diancourt L, Passet V, Nemec A, Dijkshoorn L, Brisse S. The population structure of *Acinetobacter baumannii*: expanding multiresistant clones from an ancestral susceptible genetic pool. *PLoS ONE* 5(4), e10034 (2010).
- 32 Bonnin RA, Cuzon G, Poirel L, Nordmann P. Multidrug-resistant *Acinetobacter baumannii* clone, France. *Emerg. Infect. Dis.* 19(5), 822–823 (2013).
- 33 Kaase M, Nordmann P, Wichelhaus TA *et al.* NDM-2 carbapenemase in *Acinetobacter baumannii* from Egypt. *J. Antimicrob. Chemother.* 66(6), 1260–1262 (2011).
- 34 Espinal P, Fugazza G, Lopez Y *et al.* Dissemination of an NDM-2-producing *Acinetobacter baumannii* clone in an Israeli rehabilitation center. *Antimicrob. Agents Chemother.* 55(11), 5396–5398 (2011).
- 35 Ghazawi A, Sonnevend A, Bonnin RA *et al.* NDM-2 carbapenemase-producing *Acinetobacter baumannii* in the United Arab Emirates. *Clin. Microbiol. Infect.* 18(2), e34–e36 (2012).
- 36 Bartual SG, Seifert H, Hippler C *et al.* Development of a multilocus sequence typing scheme for characterization of clinical isolates of *Acinetobacter baumannii*. *J. Clin. Microbiol.* 43(9), 4382–4390 (2005).
- 37 Kramer A, Schwelke I, Kampf G. How long do nosocomial pathogens persist on inanimate surfaces? A systematic review. *BMC Infect. Dis.* 6, 130 (2006).
- **Exhaustive review on the persistence of pathogens on inanimate surfaces.**
- 38 Adams MD, Goglin K, Molyneaux N *et al.* Comparative genome sequence analysis of multidrug-resistant *Acinetobacter baumannii*. *J. Bacteriol.* 190(24), 8053–8064 (2008).
- 39 Zanetti G, Blanc DS, Federli I *et al.* Importation of *Acinetobacter baumannii* into

a burn unit: a recurrent outbreak of infection associated with widespread environmental contamination. *Infect. Control Hosp. Epidemiol.* 28(6), 723–725 (2007).

- 40 Krizova L, Bonnin RA, Nordmann P, Nemec A, Poirel L. Characterization of a multidrug-resistant *Acinetobacter baumannii* strain carrying the *bla*_{NDM-1} and *bla*_{OXA-23} carbapenemase genes from the Czech Republic. *J. Antimicrob. Chemother.* 67(6), 1550–1552 (2012).
- 41 Gottig S, Pfeifer Y, Wichelhaus TA *et al.* Global spread of New Delhi metallo- β -lactamase 1. *Lancet Infect. Dis.* 10(12), 828–829 (2010).
- 42 Bogaerts P, Rezende de Castro R, Roisin S *et al.* Emergence of NDM-1-producing *Acinetobacter baumannii* in Belgium. *J. Antimicrob. Chemother.* 67(6), 1552–1553 (2012).
- 43 Nakazawa Y, Ii R, Tamura T *et al.* A case of NDM-1-producing *Acinetobacter baumannii* transferred from India to Japan. *J. Infect. Chemother.* 19(2), 330–332 (2013).
- 44 Hu Y, Zhang W, Liang H *et al.* Whole-genome sequence of a multidrug-resistant clinical isolate of *Acinetobacter lwoffii*. *J. Bacteriol.* 193(19), 5549–5550 (2011).
- 45 Chen Y, Cui Y, Pu F *et al.* Draft genome sequence of an *Acinetobacter* genomic species 3 strain harboring a *bla*_{NDM-1} gene. *J. Bacteriol.* 194(1), 204–205 (2012).
- 46 Zhou Z, Guan R, Yang Y *et al.* Identification of New Delhi metallo- β -lactamase gene (NDM-1) from a clinical isolate of *Acinetobacter junii* in China. *Can. J. Microbiol.* 58(1), 112–115 (2012).
- 47 Yang J, Chen Y, Jia X *et al.* Dissemination and characterization of NDM-1-producing *Acinetobacter pittii* in an intensive care unit in China. *Clin. Microbiol. Infect.* 18(12), e506–e513 (2013).
- 48 Carattoli A, Villa L, Poirel L, Bonnin RA, Nordmann P. Evolution of IncA/C *bla*_{CMY-2}-carrying plasmids by acquisition of the *bla*_{NDM-1} carbapenemase gene. *Antimicrob. Agents Chemother.* 56(2), 783–786 (2012).